

## Estimation of the risk of *Salmonella* shedding by finishing pigs using a logistic model obtained from a survey

Fablet, C., Robinault, C., Jolly, J.P., Dorenlor, V., Eono, F., Eveno, E., Labbé, A., Bougeard, S., Fravalo, P., Madec, F.

AFSSA, French Agency for Food Safety, Zoopôle Les Croix, B.P.53, 22 440 Ploufragan, France

\*corresponding author: [c.fablet@ploufragan.afssa.fr](mailto:c.fablet@ploufragan.afssa.fr)

### Abstract

An analytic epidemiological survey was carried out in 105 French farms to identify factors associated with *Salmonella* shedding by finishing pigs. This study gave out a list of 7 risk factors using a logistic model. The aim of the present survey was to validate this model on a second sample of batches of pigs in order to estimate their *Salmonella* status. The validation study was carried out from April 2003 to August 2005 on 64 finishing pig batches distinct from those used originally to generate the logistic model. In each farm, *Salmonella* shedding of a batch of pigs at the end of the finishing phase was assessed using swabs as described in the analytical study. Questionnaires were filled in with the farmer to collect data related to management routines. Blood samples from 10 growing and 10 finishing pigs were taken to assess sanitary risk factors: status vs *Lawsonia intracellularis* and Porcine Respiratory Coronavirus. *Salmonella* contamination status of a finishing room before loading, a further identified risk factor, was tested by environmental swabbing procedure. The estimated risk with the standard error, of *Salmonella* shedding was calculated using the logistic model and compared to the bacteriological *Salmonella* status of each batch. Several thresholds are proposed and sensitivity, specificity, positive and negative predictive values related to each cut-off value were calculated. A cut-off value of 0.34 maximised both sensitivity (76.9%) and specificity (68.6%) of the model. Whatever the threshold, the accuracy of the *Salmonella* non-shedding predicted status is better than the *Salmonella* shedding predicted status. In a bacteriological sampling programme, this model could be a useful tool to identify batches with low risk of *Salmonella* shedding and to focus attention on those getting a high probability for being positive.

### Introduction

*Salmonella enterica* is a frequent cause of bacterial food poisoning worldwide. Although outbreaks are frequently related to the consumption of contaminated eggs and egg products, contaminated pork products have been incriminated in human salmonellosis cases (Hald *et al.*, 1999; Van Pelt *et al.*, 2001). Contamination of pig meat is related to asymptomatic intestinal carriage of *Salmonella* by living pigs arriving at the slaughterhouse (Borch *et al.*, 1996). Furthermore, the risk of *Salmonella* contamination of the caecal contents of pigs at the slaughter line is increased for pigs belonging to batches shedding *Salmonella* at the farm (Beloeil *et al.*, 2004). Therefore, a reduction of *Salmonella* carriage at the farm level and/or the identification of at-risk batches delivered to the slaughterhouse with the implementation of segregated slaughtering procedures should help to decrease *Salmonella* contamination all along the food chain. Several epidemiological studies have been conducted to better understand circumstances associated with *Salmonella* contamination of finishing pig. Results of analytic surveys, with the determination of at-risk production practices, are used to identify critical points in farm management and constitute a basis for control programs. In analytical studies, logistic models are often used to identify risk factors. As far as they are sufficiently robust, these models could further be used in a predictive aim. To the best of our knowledge, no study was designed to assess the predictive value of a model dealing with the *Salmonella* shedding by finishing pigs. An analytic epidemiological survey was carried out in 105 French farms to identify factors associated with *Salmonella* shedding by finishing pigs (Fablet *et al.*, 2003). This study gave out a list of 7 risk factors using a logistic model. The aim of the current study was to validate this model on a second sample of batches of pigs in order to estimate their *Salmonella* shedding status.



## Material and methods

### Study design

The validation study was carried out from April 2003 to August 2005 on 64 finishing pig batches distinct from those used originally to generate the logistic model. In every farm, *Salmonella* shedding of a batch of pigs at the end of the finishing phase, housed in the same room, was assessed using swabs as described in the analytical study. Briefly, overshoes, i.e. sterile pairs of gauze socks (Sodibox, La Forêt Fouesnant, France), were used to wipe faecal material on the slatted floor of each pen. The sampling method consisted in walking on the floor wearing the overshoes. The 7 risk factors determined in the analytic study are presented **Table 1**. They were gathered by means of questionnaires and sampling. Questionnaires were filled in with the farmer to collect data related to management routines, identified as risk factors in the first study. Blood samples from 10 growing (115 days old) and 10 finishing (batch of interest) pigs were taken to assess sanitary risk factors: infection status vs *Lawsonia intracellularis* (Knittel *et al.*, 1998) and Porcine Respiratory Coronavirus. *Salmonella* contamination status of a finishing room before loading, another identified risk factor, was tested by an environmental swabbing procedure. In each pen, one sterile gauze swab (Sodibox, La Forêt Fouesnant, France) was used to wipe the bottom of the walls and the pen partitions and 1m<sup>2</sup> of the slatted floor of the pen.

### Bacteriological analyses

After use, the Sodibox soiled swabs and overshoes were placed into sterile bags and brought to our laboratory on the day they were collected. The *Salmonella* detection protocol involved four steps. Environmental swabs and overshoes were incubated 20 hours at 37°C in respectively 150 mL and 300 mL of buffered peptone water (neutralised for the post cleaning and disinfection swabs) (AES Laboratoire, Combourg, France). Following the pre-enrichment step, two selective media were used: Müller-Kauffmann Tetrathionate Broth (MKTB) and Modified Semi-Solid Rappaport Vassiliadis agar (MSRV), incubated respectively 24 hours at 42°C and 48 hours at 41.5°C. The migrated colonies of MSRV plates were isolated on Rambach agar plates and each MKTB on Xylose-Lysine-Tergitol4 (XLT4) agar plates. Both media were incubated 24 hours at 37°C. The presumptive colonies (at least one per selective media) were biochemically confirmed on Kligler-Hajna medium (AES Laboratoires, Combourg, France). All isolates were serotyped by agglutination following the Kauffman-White scheme using *Salmonella* polyvalent O and H antisera (Diagnostics Pasteur, Paris, France) (Popoff and Le Minor, 1992).

A batch was considered *Salmonella* shedding as soon as one of the overshoes tested positive. A room was considered *Salmonella*-residually contaminated as soon as one sample tested positive for *Salmonella*.

### Statistical analyses

The logistic model built in the previous analytic study is presented next:

Logit (SHEDDING) = 1,3037 FAR1 + 0,9879 FAR2 + 1,2792 FEED + 1,2150 ENVCONTAM + 1,9392 PRCV + 1,1026 Laws + 1,1658 PW - 5,5839 + residual

where FAR1: Frequency of sows' faeces removal in farrowing room, FAR2: Emptying the pit below the slatted floor between two successive batches of sows in the farrowing room, ENVCONTAM: *Salmonella* contamination of the finishing room prior to loading of a new batch of pigs, PW: Duration of the down period in the post-weaning room, FEED: Type of feeding during the fattening phase, PRCV: Infection status vs PRCV, Laws: Seroconversion against *Lawsonia intracellularis* in the second half of the fattening phase.

From this logistic model, the estimation of the probability of shedding at the end of the finishing phase was calculated according to the following formula (SAS Institute Inc., 2001):

$$p = \frac{e^{(1,X)\beta}}{1 + e^{(1,X)\beta}}$$
 where (1,X) is the matrix of the variable X and  $\beta$  the vector of the parameters estimate of X.

The estimated risk with the standard error of *Salmonella* shedding was compared with the *Salmonella* status of each of the 64 batches under study. Several thresholds (values to classify the batches having either a "low" or a "high" risk of *Salmonella* shedding) were calculated and



sensitivity, specificity, positive and negative predictive values related to each cut-off value were assessed.

## Results

Among the 64 batches included in the validation survey, 12 (18.9%) tested positive for *Salmonella*. Distribution of the estimated risk for shedding and non shedding batches is presented Figure 1. Several cut-off values can be established. Figure 2 shows the evolution of sensitivity (Se) and specificity (Sp) according to the threshold retained. Overall, Sp increased when Se decreased. The best Se is obtained for low cut-off values. Sp raised with increasing cut-off values. A cut-off value of 0.34 maximised both Se (76.9%) and Sp (68.6%) of the model. The threshold maximizing Se (92.3%) is 0.12. In this case the specificity is 41.2%. Positive and Negative Predictive Values (PPV and NPV) for 3 prevalences and 6 cut off values are presented Table 1. Whatever the prevalence, PPVs are higher for threshold 0.34, 0.45, 0.40 and NPVs are higher for 0.12, 0.24, 0.34. The threshold of 0.34 is common for higher PPV and NPV, especially for prevalence around 20 and 30%.

Figure 1: Risk estimate (bar  $\pm$  1 S.D.) and *Salmonella* status of the batches at the end of the fattening phase (64 batches, Western France, 2003-2005)

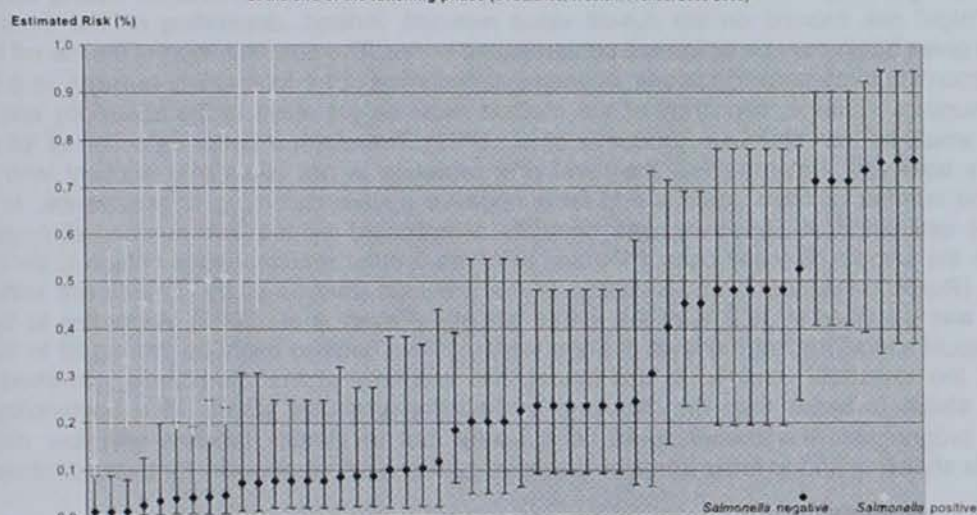


Figure 2: Sensitivity and specificity of the logistic model according to different cut-off values (64 batches - Western France, 2003-2005)

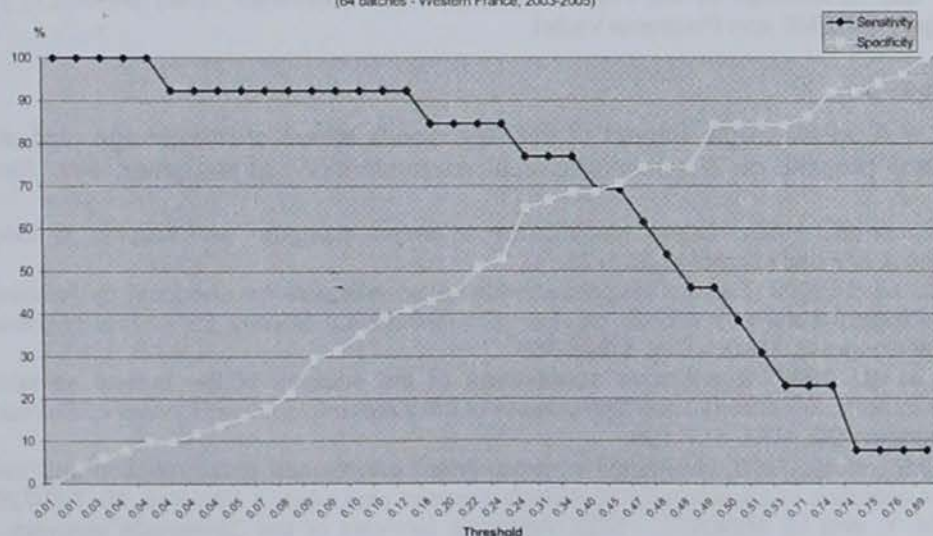


Table 1: Positive and Negative Predictive Values (PPV, NPV) according to the prevalence and the threshold retained

		Threshold		
		0.12	0.24	0.34
40 %	PPV	51.1	54.5	62.0
	NPV	88.9	83.8	81.7
30 %	PPV	40.2	43.5	51.2
	NPV	92.6	88.9	87.4
20 %	PPV	28.2	31	38.0
	NPV	95.5	93.2	92.2

## Discussion - Conclusions

The purpose of our study was to assess the characteristics of a logistic model to predict the *Salmonella* status of batches of pigs at the end of the fattening phase. To run the mathematical model, information are needed on housing and managements practices. Blood samples and swabbing are also required. These data can be obtained before shipment to the slaughterhouse and the estimate risk of *Salmonella* shedding can be calculated. Therefore adequate slaughtering procedures might be performed for "at risk" batches. The qualification of batches having either a "low" or "high" risk depend on the cut-off value retained. Indeed, depending on the threshold chosen, a given batch may be estimated contaminated or not. The determination of the cut-off level is done according to strategic decisions: economical, technical or for food safety reasons. In a food safety assurance scheme, sensitivity of the method must be preferred to the specificity and the threshold would be low (Rumeau-Rouquette *et al.*, 1993). Therefore, a better detection of "at risk" batches is achieved. Nevertheless, the level of prevalence is not taken into account with this criteria and number of false positive and false negative evolve according to prevalence. In our study, the estimation of sensitivity was certainly complicated by the low number of negative batches in the sample. In these case, PPV and NPV are 2 other recommended criteria to define a threshold (Rumeau-Rouquette *et al.*, 1993). In the previous analytic study, *Salmonella enterica* shedding was identified in 36.2 % of the tested batches (Fablet *et al.*, 2003). According to these data, we could speculate that the level of *Salmonella* positive batches might be around 20 to 35 %. Whatever the threshold retained in our model, the accuracy of the *Salmonella* non-shedding predicted status is better than the *Salmonella* shedding predicted status. In a bacteriological sampling programme, this model could be a useful tool to identify batches with low risk of *Salmonella* shedding and to focus attention on those getting a high probability for being positive.

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